Synthesis of Heterocyclic Molecules as Potential Anti-Alzheimeric Agents

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Chapter 1

INTRODUCTION

1 Alzheimer Disease (AD): An overview

Neurological disease has a destructive impact on society, healthcare system, and patients along with their care providers. Alzheimer disease (AD) is one of them and was first described by Alois Alzheimer in 1906. It is a progressive neurological disorder in the elderly people for which no cure exists.[1] It is a neurodegenerative disorder which is the main common cause of dementia.[2] This disease is responsible for the loss or decline of memory, thinking, judgement and other related cognitive skills which affect day to day activities of any person.[2] This disease is considered as major killer.[3] This disease also causes the damage or abnormal functions of nerve cells in part of the brain that is responsible for cognitive functions. According to World Health Organization (WHO), 60-70% cases of dementia are contributed by AD alone.[4] As per the report of Alzheimer's association, about 5.3 million Americans of all ages have Alzheimer's disease in 2015.[2] Approximately 44 million people worldwide having age 65 and older have AD and number of patients may increase to more than 115 million by 2050.[5] It has been observed that patient having age 65 or more survives an average of four to eight years or in some cases, a maximum of 20 years after the diagnosis of AD.[6]

1.1 Disease process, Diagnosis and treatment

There are two hypothesis reported for the diagnosis of AD.

1.1.1 Cholinergic hypothesis for alzheimer disease

This hypothesis was proposed after seeing significant loss of cholinergic neurons in the brain of AD patient. This oldest hypothesis is still used for the treatment of AD at the early stage. There is also a loss of activity of choline acetyltransferase which catalyzes the formation of acetylcholine in the presynaptic neurons resulting in decreased neurotransmission and cognitive dysfunction.[7] Acetylcholine is an important neurotransmitter which is involved in learning and memory. Normal aging causes a slight decrease in acetylcholine concentration, causing periodic forgetfulness. However in AD, the concentration can be decreased by as much as ninety-percent, resulting in significant loss of memory and declining of behavioral attributes. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) play an important role in the reduction of

acetylcholine by hydrolyzing acetylcholine to choline and acetate. The cholinesterases are enzymes in a family of serine hydrolases because of their ability to hydrolyze substrate using an active site nucleophillic serine residue. The super family of serine hydrolases belongs to a large group of proteins which are involved in many important physiological processes e.g. blood coagulation,[8] digestion,[9] and neurotransmission,[10] and therefore, many of these enzymes are linked to various diseases such as pancreatitis, thrombosis and Alzheimer's disease (AD). Therefore, cholinesterases are attractive targets for drug discovery.

These cholinesterases consist of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and are responsible for the breakdown of cholinergic neurotransmitters acetylcholine and butyrylcholine, respecively. Both AChE and BChE have known structures, but only the former has a well-established function. The ACHE is found in most of the tissues like neuromuscular junctions,[11] brain cholinergic synapses,[12] autonomic ganglia[13] and red blood cell membranes.[14] This enzyme is known as a modulator of neurotransmission which hydrolyses neurotransmitter acetylcholine (Ach) synthesized from acetyl coenzyme A (AcCoA). This coenzyme is synthesized from glucose and choline by the catalytic action of choline acetyltranferase enzyme (ChAT) and stored into synaptic vesicles. From the synaptic vesicles, Ach is released to presynaptic nicotinic (N) and muscarine type 2 (M_2) receptors which further release this Ach to postsynaptic M_1 receptor. During Ach transfer to post synaptic neurons, the AChE breaks down the Ach which is left in the synaptic gap into choline and acetate (Figure 1.1). These molecules are again transferred into presynaptic neurons for Ach synthesis[15]. Several AChE functions have also been reported such as cellular differentiation and tumorigenesis,[16] and apoptosis.[17,18] Unattended release of Ach results in continuous stimulation of receptors that causes symptoms like confusion, vomiting, convulsion and respiratory failure.[19] On the contrary, lack of release of Ach reduces receptor stimulation resulting in cognitive impairment, one of the important cause of AD.[20]

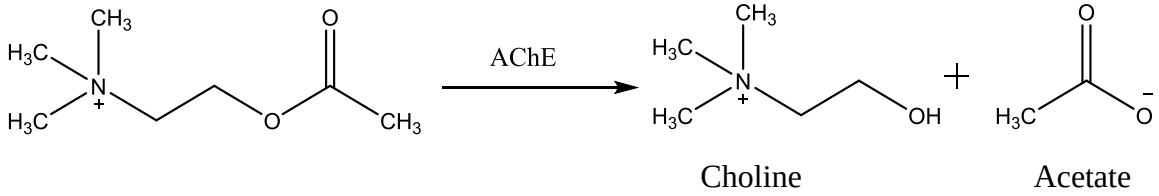


Fig. 1.1: Acetylcholinesterase (AChE) hydrolyzing acetylcholine to Choline and Acetate

Butyrylcholinesterase (BuChE), also known as pseudocholinesterase, is a serine hydrolase which catalyzes the hydrolysis of ester of choline including butyrylcholine, succinylcholine and

acetylcholin.[21] In contrast to AChE which is predominantly present in the brain, BChE is more active in the peripheral tissue than in the brain[22] and mostly found in serum and glial cells. It is also present in neurons.[23,24] AChE exhibited specificity towards the neurotransmitter acetylcholine,[25] whereas BuChE catalyzes the hydrolysis of a wide variety of choline and non-choline esters[26] such as acetylcholine same as ACh,[27] succinylcholine,[28] cocaine[29] and aspirin.[30] Due to this function it plays an important role in neurotransmission, anaesthesia and drug abuse.

Both AChE and BuChE are encoded on different chromosomes, but they exhibit 65% amino acid sequence homology[31] and they contain a catalytic triad which is composed of the amino acids serine (Ser), histidine (His) and glutamic acid (Glu) which are located at the bottom of a 20 Å gorge.[32,33] Because of the important role of cholinesterases in human body, these enzymes play important role in several disease, and are considered attractive targets in the field of drug discovery for different kind of disease including AD.[34,35]

1.1.2 Amyloid hypothesis

According to ‘‘amyloid hypothesis’’, the AChE produces secondary non-cholinergic functions which enhance the formation and deposition of beta-amyloid (Aβ) in the form senile plaques and hyperphosphorylated twisted tau protein in the form of neurofibrillary tangles in the brain of AD affected individuals.[36] There are various methods of diagnosis of AD in the brain like functional MRI, positron emission tomography (PET), CT scan, etc. [37,38]

1.2 Therapeutic targets for AD

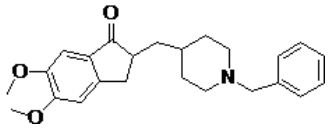
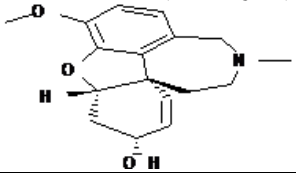
Out of the different drug targets available for the treatment of AD, the AChE and BuChE inhibitors were widely explored. These enzymes are responsible for the breakdown of acetylcholine to choline and acetate hence resulting in loss of acetylcholine in the neurons. Healthy neurotransmission is essential for the cognitive function; therefore by inhibiting AChE and BuChE, the level of acetylcholine can be restored resulting in improved cognitive functions. Among the other targets, BACE and ϒ-secretase are responsible for formation of β-amyloid through the abnormal cleavage of amyloid precursor protein (APP). Hence, their inhibition is also considered important target for the treatment of AD.[39]

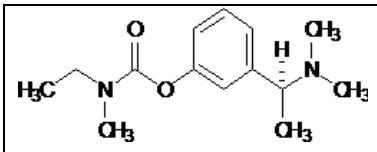
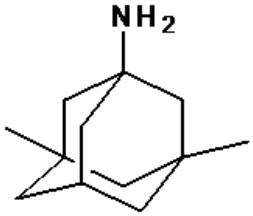
MAO B inhibitor is a potential target for the treatment of AD. This MAO B enzyme leads to the formation of aldehyde and hydrogen peroxide by the catabolism of neurotransmitter containing amino group. Hence the increase in MAO B activity results in more production of

hydrogen peroxide which leads to oxidative stress and in turn is responsible for neurodegeneration. But none of them permanently cure this disease. The Food and Drug Administration (FDA) had approved two types of medication for the treatment of AD. The first type is called a cholinesterase inhibitor, which hinders the enzyme responsible for the breakdown of acetylcholine in the brain.[40] Currently, three cholinesterase inhibitors commonly prescribed: donepezil, galantamine, and rivastigmine (Table 1). The function of these drugs is to support communication between nerve cells through increasing the concentration of Ach.

In addition to cholinesterase inhibitors, a medication called Memantine (Table 1.1), which regulates the activity of glutamate, has also been approved for the treatment of AD.[41,42] The Glutamate is an excitatory neurotransmitter involved in learning and memory. Over stimulation of nerves by glutamate may be the cause of neuro-degeneration. The glutamate binds to N-methyl-D-aspartate (NMDA) receptors on the surface of brain cells. Memantine functions by blocking the NMDA receptors and therefore, protecting the nerves from excessive glutamate stimulation. This drug is used for the treatment of moderate to severe AD. Several small heterocyclic molecules as AchE/BuChE inhibitor were also synthesized by several research groups. Only some of them are approved for the treatment of AD at the early stages and some are under pre-clinical as well as clinical studies. Some molecules that do not reach up to clinical trial, and they need some modifications in their structures. On the basis of ongoing research in this field, we have synthesized chromene, thiazole and triazole based heterocyclic molecules as potential anti alzheimeric agents. After their synthesis, all derivatives were evaluated against AChE and BuChE.

Table 1.1: Drugs, their action and their adverse effects*

Drug Name	Action	Adverse Effects
Donepezil (Aricep) 	Prevents the breakdown of acetylcholine (ACh) by inhibiting the action of acetylcholinesterase	CNS: headache, seizures, insomnia, fatigue, aggression CV: chest pain, hypertension, atrial fibrillation GI: nausea, vomiting, GI bleeding Metabolic: Weight loss, dehydration
Galantamine (Razadyne) 	Prevents the breakdown of acetylcholine and stimulates receptors to release excess ACh	CNS: depression, dizziness, fatigue, insomnia CV: bradycardia, AV block GI: diarrhea, nausea, anorexia, abdominal pain Hematologic: anemia
Rivastigmine (Exelon)	Prevents the breakdown	CNS: headache,dizziness,

	of acetylcholine by inhibiting the enzymes that degrade Ach	confusion,nervousness, paranoia,malaise CV: hypertension, chest pain, edema Musculoskeletal: back pain, bone fractures Respiratory: bronchitis, cough
Memantine (Namenda) 	Blocks glutamatergic (NMDA) receptors and regulates the action of glutamate	CNS: stroke, aggressiveness, agitation, fatigue, confusion, pain, syncope CV: heart failure, edema GI: anorexia, constipation, nausea, vomiting Skin: Rash

*Taken from book; Neuroscience, 3rd edition by D. Purves et al, 2004

CNS: central nervous system; CV: cardiovascular; GI: Gastrointestinal

The adverse effect of the drugs on patients along with other limitations assures that there is a requirement for novel compounds that can be developed into better drug for the cure of AD. This prompted us to take this work and synthesize new series of heterocyclic compounds which on *in silico* and *in vitro* study can give lead molecules. Therefore, the following three are objectives of my thesis:

1.3 Objectives

Scheme 1: *Design, synthesis and evaluation of 3-[2-(4-phenylthiazol-2-ylamino)-acetyl]-chromen-2-one derivatives as AChE and BuChE inhibitors*

Scheme 2: *Design, synthesis and evaluation of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives as AChE and BuChE inhibitors*

Scheme 3: *Design, synthesis and evaluation of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives as AChE and BuChE inhibitors*

The thesis will be organized into five Chapters. The Chapter 1 discusses the overview of Alzheimer Disease (AD). Chapters 2, 3 and 4 describe the synthesis of novel heterocyclic molecules along with their evaluations against the AChE and BuChE. Chapter 5 discusses conclusion and future perspectives.

Chapter 2

Design, synthesis and evaluation of 3-[2-(4-Phenyl thiazol-2-ylamino)-acetyl]-chromen-2-one derivatives as AChE and BuChE inhibitors

2.1 Introduction

Coumarins are the classes of heterocyclic molecule and constructed by benzene fused with α-pyrone ring (Figure 2.1). These are naturally occurring phytochemicals in many plant species with a wide range of biological activities such as anti-inflammatory,[43] anti-tumor,[44] hepatoprotective, anti-allergic, anti-HIV-1, antiviral, antifungal, antimicrobial, antiasthmatic,[45] anti-oxidant,[46] antinociceptive,[47] anti-diabetic and antidepressant effects.[48] In recent years, coumarin derivatives have attracted attention because they possess a wide range of biological activities associated with neurological disorders, especially for Alzheimer's disease (AD). Studies on coumarin analogues have also shown that naturally occurring as well as the chemically synthesized coumarin analogs exhibit potent AChE inhibitory activity.[49] Furthermore, functionalization of the aromatic center of coumarins has led to the development of novel analogs that are capable of inhibiting beta-amyloid Aβ aggregation.[50] Studies have also shown that the anti-amnestic and the memory restorative functions of the coumarin derivatives in different experimental models of amnesia.[51] Coumarin derivatives also provide protection to neurons against Aβ-induced oxidative stress and free radicals.[52] The compounds with coumarin as heterocyclic moiety have appreciable AChE inhibitory activity. Coumarin ring seems to be essential for the optimal activity, and when it was replaced by related structural moiety such as chromone, the AChE inhibitory activity was lost.[53] The length of linker which connects coumarin with other heterocycle moiety and hydrophobic interaction between the latter with catalytic site are important parameters for influencing AChE inhibitory activity[53] (Figure 2.2). Most of the studies have reported that compounds with tetramethylene or phenyl linker are more potent AChE inhibitors.[53] Literature shows that the 3rd or 4th position of coumarin moiety is the most favorable for linking catalytic site interacting moiety for obtaining potent dual site interacting AChE inhibitors.[53] Such substitution at 6th or 7th position generally does not increase the potency of compounds. When bulkier substituents are present at 6th and 7th positions of the coumarin the inhibitor activity towards AChE were decreased.[53]

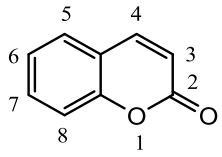


Figure 2.1: Structure of coumarin with different positions

2.2 Design of 3-[2-(4-Phenyl thiazol-2-ylamino)-acetyl]-chromen-2-one derivatives and their preliminary *in-silico* analyses as AChE and BuChE inhibitors

Preliminary docking studies of fragments had indicated that coumarin and thiazole interact with the active site of AChE and BuChE. Therefore, the compounds with these moieties were designed and synthesized.

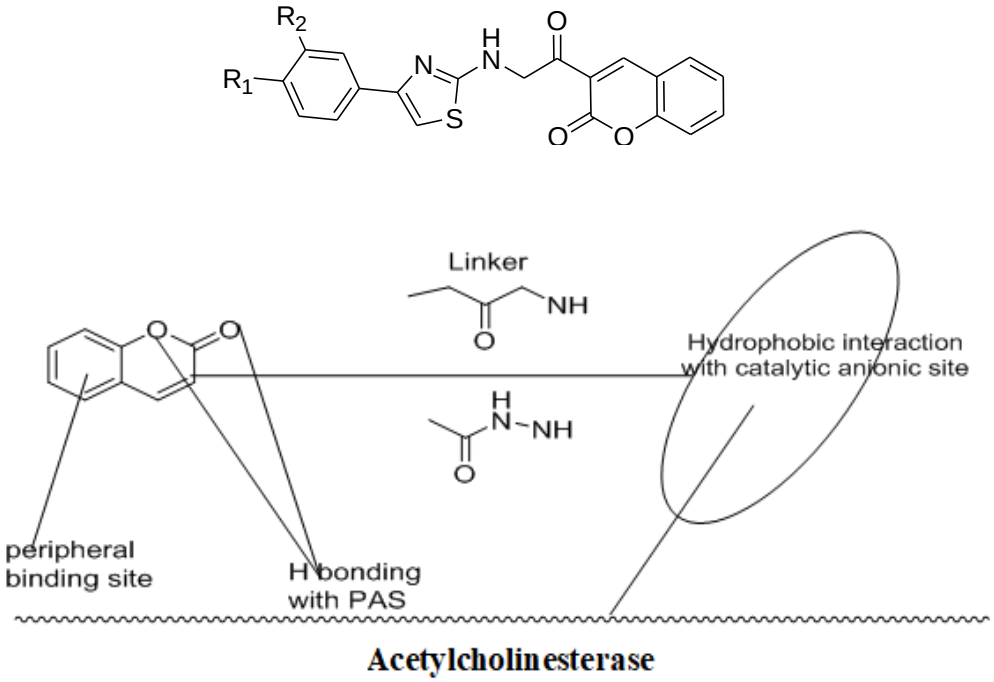


Figure 2.2: Coumarin-thiazole conjugate used for design and synthesis potential lead molecules (shown above)

2.3 Experimental Section

The synthesis of 3-[2-(4-Phenyl thiazol-2-ylamino)-acetyl]-chromen-2-one derivatives (**8a-8l**) has been achieved by Scheme 1. The synthesized compounds were characterized by FTIR, ^{1}H and ^{13}C NMR, and elemental analysis.

Synthesis of 3-acetyl-2H-chromen-2-one (3)

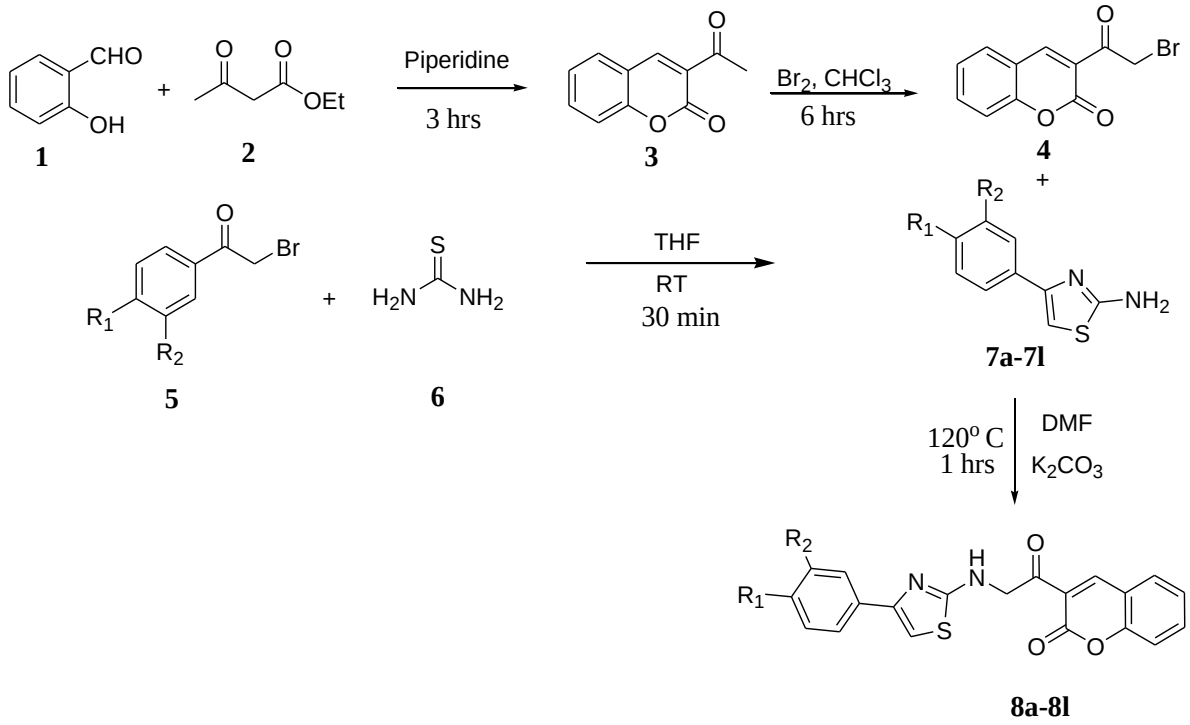
A solution of ethyl acetoacetate (**2**, 3.0 mL, 23.5 mmol) in ethanol (15 mL) was taken in a round bottom flask (250 mL). The solution was kept at 0^oC and further piperidine (0.2 mL, 2.0 mmol) was added. The resulting mixture was stirred at 0^oC for 5 min followed by addition of salicylaldehyde (**1**, 2.5 mL, 23.5 mmol). The reaction mixture was allowed to come at room temperature and stirred for 3 hrs. The progress of the reaction was monitored by TLC (hexane:ethylacetate, 7:3). A crude yellow solid was obtained. The reaction mixture was filtered and product was washed with ice cold water-ethanol (7:3, v/v). The product was further recrystalized by using ethanol. Yellow pure solid of 3-acetyl-2H-chromen-2-one was obtained which was characterized by FTIR, NMR and melting point.

Yield: 4.16 gm (94%); Mp.: 120-121^oC (lit.[53] Mp. 120^oC); IR (KBr): 3460, 3443, 3116, 2757, 1627, 1525, 1487, 1210, 1153, 846, 593 cm^{-1}; ^1H NMR (DMSO-d^6, 400 MHz): δ 2.49 (3H, s, CH_3), 7.40-7.96 (4H, m, ArH), 8.64 (1H, s, H of pyran ring); ^{13}C NMR (DMSO-d^6, 100 MHz): δ 30, 116,118, 124, 125, 131, 134, 147, 155, 158, 195; Anal. calc. for $C_{11}H_8O_3$: C, 70.21; H, 4.29; O, 25.51; found C, 69.44; H, 4.24.

Synthesis of 3-(2-bromoacetyl) chromen-2-one (4)

A solution of 3-acetyl-2H-chromen-2-one (**3**, 28.2 g, 150 mmol) in 150 mL of alcohol free chloroform was taken in round bottom flask (250 mL). The solution was kept at 0^oC and further Br_2 (7.6 mL in 20 mL $CHCl_3$, 150 mmol) was added with the help of dropping funnel. After addition of all bromine the reaction mixture was allowed to come at room temperature and stirred vigorously for 6 hrs. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was heated on water bath for 15-20 min to expel most of the HBr and for decomposition of by products. Then the reaction mixture was cooled. Solid product was separated which was filtered and washed with ether. The product was further recrycstalised by using glacial acetic acid. Solid colorless needle shaped crystal was obtained which was characterized by IR, NMR and melting point.

Yield: 34.26 gm (85.5%); Mp.: 162-163^oC (lit.[54] Mp. 162^oC); IR (KBr): 3435,1599, 1532, 1518, 1483, 1341, 1023, 846, 774, 669, 569, 474 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 4.75 (2H, s, CH_2), 7.36-7.72 (4H, m, ArH), 8.64 (1H, s, H of pyran); Anal. calc. for $C_{11}H_7BrO_3$: C, 49.47; H, 2.64; found C, 48.92; H, 2.60.



Comp. No.	R_1	R_2	Comp. No.	R_1	R_2
8(a)	H	H	8(g)	NO_2	H
8(b)	Fl	H	8(h)	CN	H
8(c)	Cl	H	8(i)	H	Br
8(d)	Br	H	8(j)	H	NO_2
8(e)	CH_3	H	8(k)	H	OCH_3
8(f)	OCH_3	H	8(l)	Cl	Cl

Scheme 1: 3-[2-(4-Phenyl thiazol-2-ylamino)-acetyl]-chromen-2-one derivatives

Synthesis of 2-amino phenyl thiazole and its derivatives (7)

A solution of substituted phenacyl bromide (**5**, 4.00 gm, 20.0 mmol) in 6 mL THF was taken in round bottom flask (100 mL). The solution was kept at room temperature and added thiourea (**6**, 1.83 g, 24.0 mmol). The reaction mixture was stirred at room temperature for 30 min. The progress of reaction mixture was monitored by TLC (hexane:ethylacetate, 7:3). After completion of reaction the solid product was separated, which was filtered and washed with water. The products were further recrystailsed by using ethanol. Solid product were dried and characterized by FTIR, NMR and melting point. The characterization data of **7a**:

Yield: 3.35 gm (95%); Mp.: 147-148^{o}C (lit.[55] Mp. 151^{o}C); IR (KBr): 3435, 3252, 3154, 2920, 2852, 1600, 1520, 1481, 1440 1333, 1215, 1072, 912, 846, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz):

δ 5.12 (2H, bs), 6.75 (1H, s), 7.33 (1H, d, *J* = 7.6 Hz), 7.42-7.38 (2H, m), 7.80 (2H, d, *J* = 7.2 Hz); Anal. calc. for $C_9H_8N_2S$: C, 61.34; H, 4.58; N, 15.90; found C, 61.44; H, 4.60; N, 15.94.

Synthesis of 3-[2-(3-phenylcycclopenta-1,3-dienylamino-acetyl]chromen-2-one derivatives (8)

A solution of phenyl thiazole-2-amines (**7a**, 500 mg, 2.84 mmol) in 3 mL DMF were taken in round bottom flask (50 mL) then added 3-(2-bromoacetyl) chromen-2-one (**4**, 758 mg, 2.84 mmol) and K_2CO_3 (100 mg, 0.72 mmol). The reaction mixture was stirred at 120^{o}C for 1 h and the progress of reaction was monitored by TLC (hexane:ethylaceate, 7:3 v/v). After completion of reaction the reaction mixture was quenched by water and extracted with ethylacetate (3×50 mL). The organic layer was washed with water (3×50 mL) and dried over anhydrous sodium sulphate and was concentrated under reduced pressure. The residue obtained was purified by silica gel (60-120 mesh) column chromatography using petroleum ether-ethylacetate (8:2) as a mobile phase to give the pure product, **8a**.

Yield: 458 mg (67%); Mp.: 190-195^{o}C; IR (KBr): 3436, 1705, 1604, 1532, 1518, 1483, 1442, 1332, 1148, 1039, 845, 714 cm.$^{-1}$; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.21 (2H, s, CH_2), 7.07 (1H, s, thiazole), 7.11-8.53 (9H, m, ArH), 8.68 (1H, s, H of pyran), 12.44 (1H,bs, NH); ^{13}C NMR (DMSO-d^6, 100 MHz): δ 32, 102, 111, 113, 118, 124, 125, 129, 131, 136, 147, 150, 155, 159, 168, 195; Anal. calc. for $C_{20}H_{14}N_2O_3S$: C, 66.28; H, 3.89; N, 7.73; found C, 65.98; H, 3.68; N, 7.68.

***In-vitro* and docking studies of 3-[2-(3-phenylcycclopenta-1,3-dienylaminoacetyl]chromen-2-one derivatives (8a-8l)**

Acetylcholinesterase (AChE, E.C. 3.1.1.7, from *Electrophorus electricus*), butylcholinesterase (BChE, E.C. 3.1.1.8, from equine serum), acetylthiocholine iodide, butyrylthiocholine iodide and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. Phosphate buffer saline (PBS) was procured from HiMedia. All the test molecules were solubilized in DMSO and were diluted further with PBS (pH 8) such that 8 mM stock concentration is achieved in a solvent having 8% DMSO.

Inhibition of acetyl-cholinesterase (AChE) and butyl-cholinesterase (BuChE) activity assay

The molecules were tested for AChE and BuChE inhibitory activity according to the method previously described by Najafi et al with some modifications.[55a] Enzyme inhibition assay was performed in a 96-well plate by using DTNB method. Briefly, 25 µL AChE/BuChE (25 mU in 100 µM PBS) was incubated with 75 µL DTNB (in 100 µM PBS, having 600 µM $NaHCO_3$) for 5 min at room temperature. To this, 25 µL of test compounds (1 – 1000 µM), and 50 µL PBS (pH 7.4) were added. Reaction mixture was then incubated for 15 min at room temperature. Reaction

was initiated by adding 25 µL of acetylthiocholine iodide and butylthiocholine (75 mM in PBS) for AChE and BuChE inhibitory assay, respectively. Change in absorbance was recorded spectrophotometrically during the experimental duration of 4 min at 412 nm by using UV-spectrophotometer. A blank reaction was run simultaneously, which was having 25 µL solvent (1% DMSO) in place of drugs. Percent inhibition of AChE/BuChE activity was calculated by using following equation

$$\%\text{AChE/BuChE inhibition} = \frac{(Absorbance\ of\ control - Absrbance\ of\ test) * 100}{Absorbance\ of\ control}$$

Docking studies target molecules against acetyl-cholinesterase (AChE) and butyl-cholinesterase (BuChE)

Geometries of the structures of 3-[2-(4-Phenyl thiazol-2-ylamino)-acetyl]-chromen-2-one intermediates and derivatives (3, 4, 7a-7l & 8a-8l; Refer Section 2.3) were optimized at the level B3LYP/STO-3G using Gaussian 09 quantum chemistry software. The structures were verified using vibrational frequencies. Crystal structure of the protein AChE (PDB Id: 1EVE) was downloaded from protein data bank (PDB: *www.rcsb.org*). Though many structures of AChE are available, but the above structure is from *Tetronarce californica* organism. The *in vitro* experiment was also carried on enzyme from the same organism. Similarly for BuChE structure, 4PTK (PDB ID) was used.

Before docking the ligand molecules and enzymes were prepared through Schrödinger's 'ligprep' and Protein preparation modules. Docking was performed using Glide module of Schrodinger software. Docking is the finding the correct orientation of conformation of small ligand molecule inside the binding pocket of protein molecule (macromolecule). This orientation of conformation is according to shape and electrostatic complementarity between ligand and protein which provides the maximum favorable (complementary) interactions. It can be divided mainly into two parts: search method (generation of different conformation and orientation (poses) ligand in binding pocket), and scoring based on interaction energy of particular pose means how good this pose is.

2.4 Results

The inhibition activity (IC_{50}) of 3-[2-(3-phenylcycclopenta-1,3-dienylaminoacetyl]chromen-2-one intermediates and derivatives (3, 4, 7a-7l & 8a-8l; Refer Section 2.3) against AChE & BuChE are given in Figure 2.3. The IC_{50} and docking scores against these two enzymes were provided in Table 2.1.

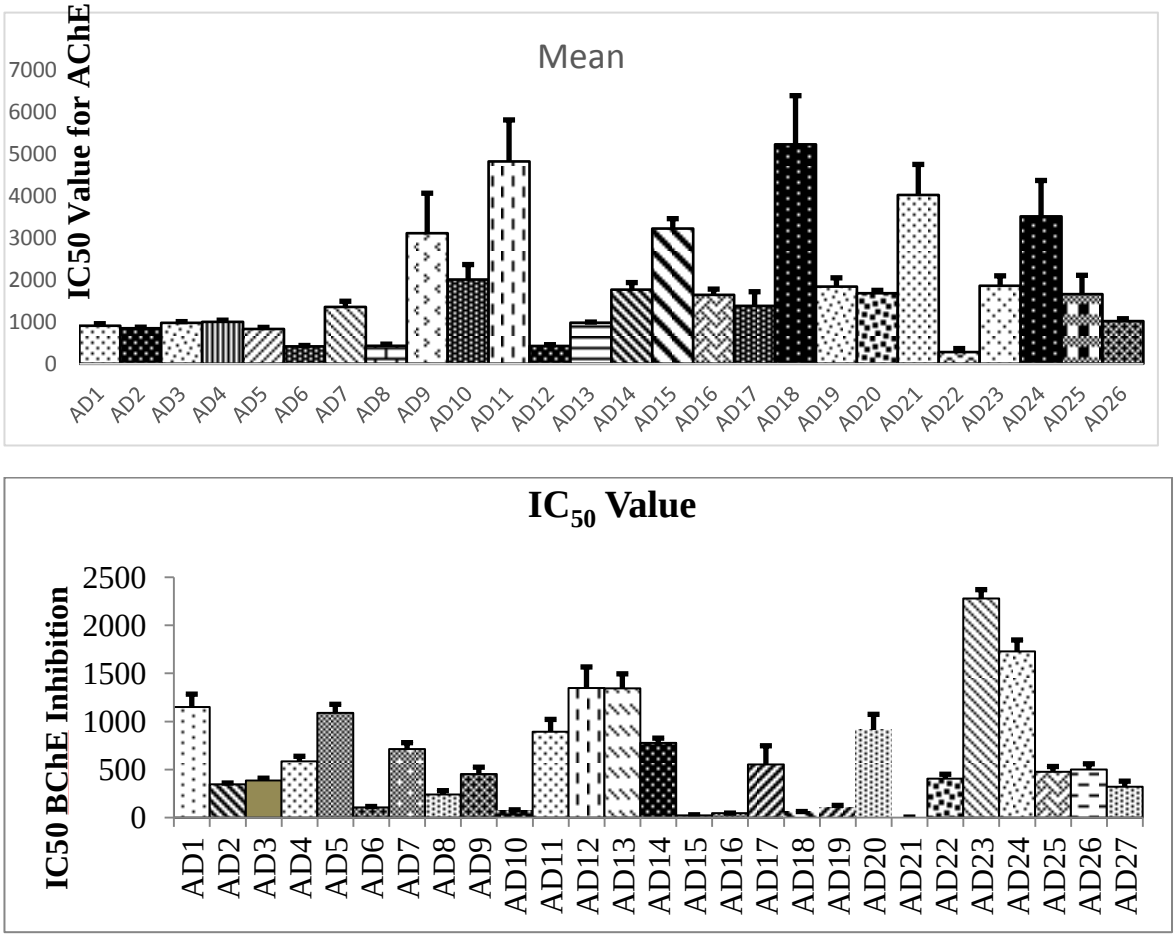


Figure 2.3: Inhibition activity (IC_{50}) of 3-[2-(3-phenylcycclopenta-1,3-dienylaminoacetyl]chromen-2-one intermediates and derivatives against ACHE and BuChE

Link between number in above Figure with molecules represented in Scheme 1

AD 1 = 3	AD 6 = 8a	AD 11 = 7d	AD 16 = 8j	AD 21 = 7i	AD 26 = 8f
AD 2 = 4	AD 7 = 8c	AD 12 = 8d	AD 17 = 8k	AD 22 = 7l	
AD 3 = 7a	AD 8 = 8e	AD 13 = 7g	AD 18 = 8i	AD 23 = 7h	
AD 4 = 7e	AD 9 = 7b	AD 14 = 8g	AD 19 = 8l	AD 24 = 7f	
AD 5 = 7c	AD 10 = 8b	AD 15 = 7j	AD 20 = 7k	AD 25 = 8h	

Table 2.1 The IC_{50} value and docking score of target compounds **3, 4, 7a-7l & 8a-8l** against AChE and BuChE.

	IC_{50} (μM)		SI	Docking Score	
	AChE	**BuChE**		**AChE**	**BuChE**
3	906.31 ± 49.13	1151.1 ± 133.90	0.78	Inactive	Inactive
4	846.33 ± 25.29	346.68 ± 13.15	2.44	-7.739	-5.856
7a	975.48 ± 29.18	385.69 ± 24.41	2.53	-5.989	-5.624
7b	3112.81 ± 953.42	453.61 ± 71.11	6.86	-5.989	-5.624
7c	829.12 ± 38.17	1089.97 ± 89.12	0.76	-5.916	-5.529
7d	4824.25 ± 989.91	893.92 ± 128.12	5.39	-5.916	-5.765
7e	998.97 ± 40.31	585.43 ± 52.83	1.71	-5.878	-5.840
7f	3514.13 ± 856.19	1730.01 ± 118.59	2.03	-5.539	-5.144
7g	978.85 ± 14.83	1345.52 ± 150.87	0.73	-5.680	-5.044
7h	1859.65 ± 237.09	2280.80 ± 91.71	0.81	-6.287	-6.342
7i	4024.77 ± 728.16	3.54 ± 1.64	1136.00	-6.364	-5.828
7j	3223.9 ± 236.21	23.24 ± 6.00	138.71	-5.510	-5.230
7k	1681.54 ± 68.37	912.14 ± 162.74	1.84	-7.739	-5.856
7l	278.44 ± 83.34	405.56 ± 45.72	0.69	-6.128	-5.937
8a	414.27 ± 21.57	106.25 ± 9.29	3.89	-8.830	-9.077
8b	2008.17 ± 357.47	76.41 ± 4.60	26.28	-9.262	-8.620
8c	1354.68 ± 135.84	713.61 ± 66.48	1.89	-10.97	-7.878
8d	423.78 ± 30.84	321.49 ± 57.75	1.32	-9.642	-6.783
8e	427.83 ± 14.83	240.69 ± 39.62	1.78	-8.804	-7.014
8f	1016.83 ± 56.50	500.8 ± 59.22	2.03	-7.261	-8.732
8g	1767.56 ± 167.81	777.81 ± 49.08	2.27	-7.620	-7.595
8h	1659.79 ± 449.63	476.89 ± 54.96	3.48	-7.964	-8.490
8i	**5231.54 ± 1160.8**	**61.64 ± 1.67**	**84.87**	**-9.165**	**-7.990**
8j	**1642.76 ± 136.93**	**46.47 ± 0.37**	**35.35**	**-6.961**	**-8.893**
8k	1379.91 ± 337.62	553.59 ± 194.15	2.49	-8.151	-9.692
8l	1839.56 ± 209.44	107.32 ± 19.73	17.14	-9.620	-8.570

Chapter 3

Design and synthesis of N-Benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives as AChE and BuChE inhibitors

3.1 Introduction

Thiazole is an important heterocyclic molecule with five-membered structures having formula, C_3H_3NS (Figure 3.1). This molecule possesses both an electron accepting (C=N) and electron donating group (-S-) moieties. This ring is present in many natural and synthetic products with a broad range of biological applications. This ring containing vitamin B_1 (thiamine) helps in the normal functioning of the nervous system by its role in the synthesis of acetylchloline.[56] These derivatives have been successfully used in the past as potential neuroprotective agents.[57] Tetrahydrobenzothiazole,[58] phenolic thiazole[59] and benzothiazole[60] are well known for their neuroprotective nature. Literature showed that thiazole–triazole linked derivatives have shown potent anti-alzheimeric activity.[61] Therefore, we have synthesized thiazole–triazole conjugates as potential AChE/BuChE inhibitor.

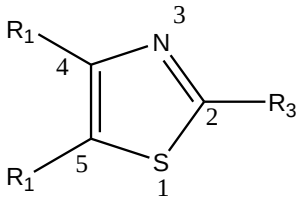
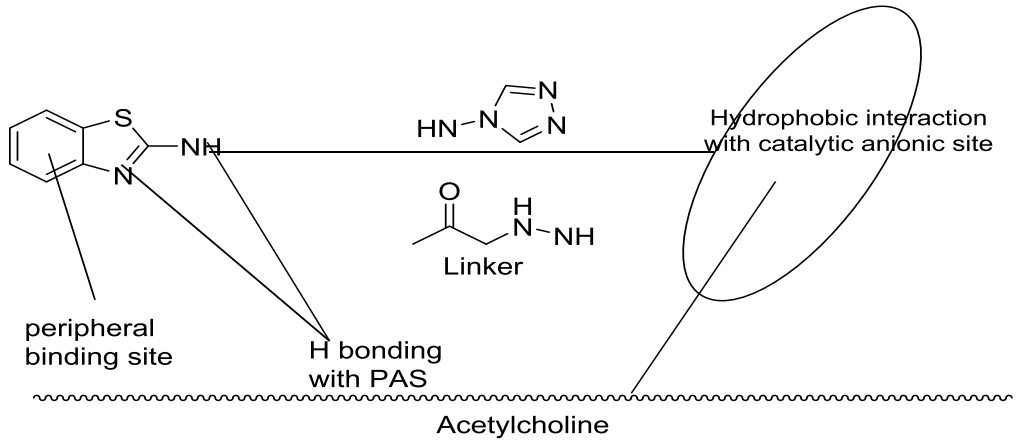


Figure 3.1: Structure of Thiazole

3.2 Design of N-Benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives and their preliminary *in-silico* analysis of moieties as AChE and BuChE inhibitors



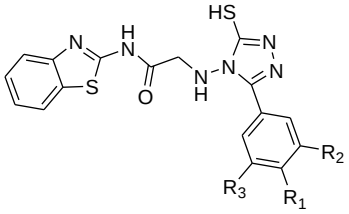
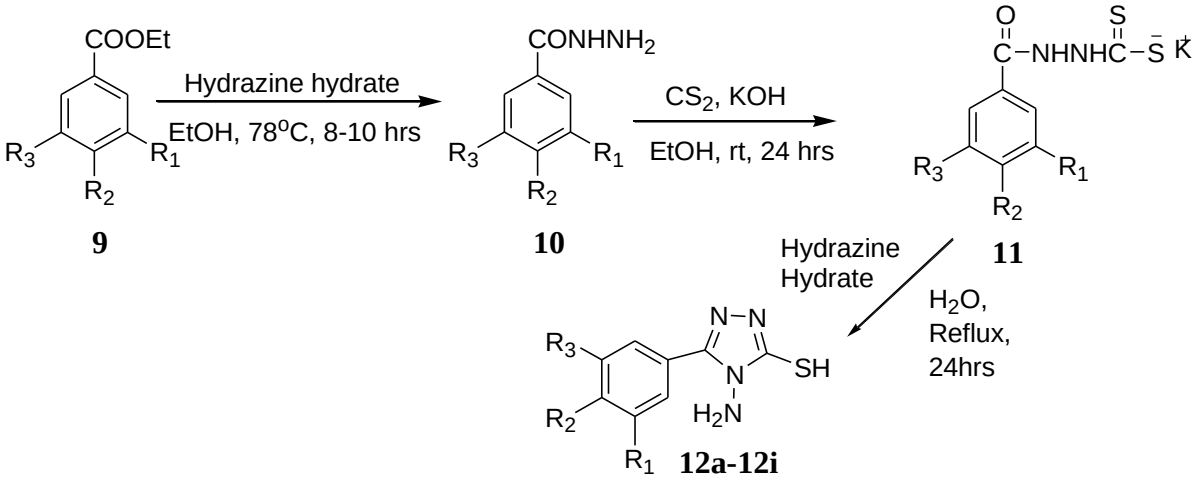


Figure 3.2: Benzothiazole-triazole conjugate used for design and synthesis potential lead molecules (shown above)

3.3 Experimental Section

The synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (**16a-16i**) has been achieved by schemes 2 and 3. The synthesized compounds were characterized by FTIR, ^{1}H and ^{13}C NMR, and elemental analysis.



Scheme 2

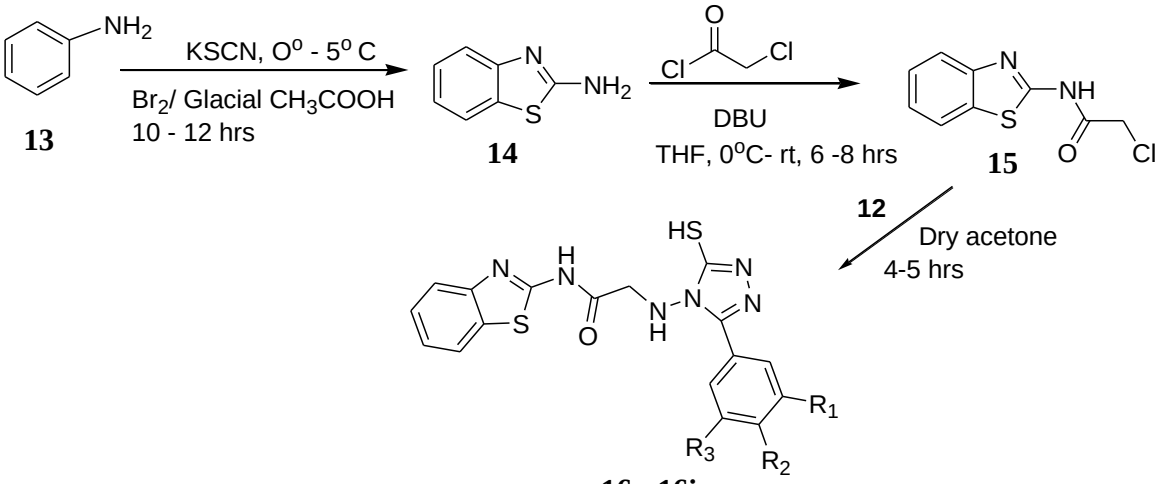
Synthesis of Substituted triazole

The synthesis of substituted triazole involves three step processes and were synthesized by well known procedure.[62]

Synthesis of benzohydrazide (10a)

A solution of substituted aromatic esters (**9**, 14.2 mL, 100 mmol) in 30 mL ethanol were taken in round bottom flask (100 mL). To this solution hydrazine hydrate (4.8 mL, 100 mmol) was added drop wise with stirring. The resulting mixture was allowed to reflux for 6 h and the progress of reaction was monitored by TLC (ethyl acetate:hexane, 1:1 v/v). After the completion of reaction excess ethanol was distilled out and the contents were allowed to cool. White needle shaped

crystal was obtained which was filtered, dried and used for next step. By using same methodology substituted benzohydrazide (**10b-10i**) were synthesized.



16a-16i

Comp. No.	R_1	R_2	R_3	Comp. No.	R_1	R_2	R_3
16(a)	H	H	H	16(f)	H	CH_3	H
16(b)	H	F	H	16(g)	H	OCH_3	H
16(c)	H	Cl	H	16(h)	H	NO_2	H
16(d)	H	Br	H	16(i)	OCH_3	OCH_3	OCH_3
16(e)	Br	H	H				

Scheme 3: N-Benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives

Synthesis of potassium 2-benzoylhydrazine-1-carbodithioate (11a)

A solution of KOH (8.41 gm, 150 mmol) in 200 mL of absolute ethanol was taken in round bottom flask (500 mL). Further substituted aryl acid hydazide (**10**, 13.6 gm, 100 mmol) were added to this solution. The reaction mixture was stirred vigorously till clear solution was obtained. After dissolution, CS_2 (9.0 mL, 150 mmol) was added in small portion with constant stirring. The reaction mixture was stirred continuously for a period of 24 h. After completion of reaction, ether was added to the reaction mixture and stirred vigorously for 30 min. The solid salt was precipitated which was filtered and dried on reduced pressure. The potassium salt thus obtained was in quantitative yield and was used in next step without further purification.

Synthesis of substituted 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (12a-12i)

A suspension of substituted potassium 2-benzohydrazine-1-carbodithioate (**11**, 2.5 g, 10 mmol) in 10 ml water were taken in round bottom flask (100 mL). Further, hydrazine hydrate (1.5 mL, 30 mmol) was added to this solution and the reaction mixture was set for refluxing. The reaction mixture was refluxed for 24 h and during refluxing the color of reaction mixture was

changes to green with the evolution of hydrogen sulfide gas. A homogeneous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water (100 ml). On acidification with concentrated hydrochloric acid, the required substituted triazole was precipitated out. It was filtered, washed thoroughly with cold water, and recrystallized from ethanol. The formed compounds were characterized by FTIR, NMR and melting point. The data of **12a** is given below.

Yield: 1.87 gm (97%); Mp.: 195-196^{o}C (lit[62] 196^{o}C); IR (KBr): 3241, 3011, 2943, 1615, 1508, 1350, 1187, 1160, 1073, 970, 846, 728, 691 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.35 (1H, s), 7.50- 8.03 (5H, m, ArH); ^{13}C NMR (DMSO-d_6; 100MHz): δ 122, 126, 128, 129, 132, 160, 177. Anal. calc. for $C_8H_8N_4S$: C, 49.98; H, 4.19; N, 29.14; S, 16.68; found C, 49.59; H, 4.08; N, 28.91; S, 16.59

Synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (15)

A solution of 2-aminobenzothiazole[63] (**14**, 5 g, 33.3 mmol) in 15 mL tetrahydrofuran (THF) were taken in round bottom flask (100 mL). Further, DBU (1 mL, 6.6 mmol) was added to the solution. The reaction mixture was kept at 0^{o} C. A dropping funnel was fitted to the flask, and a solution of chloroacetylchloride (3.2 mL in 2 mL THF, 40 mmol) was taken in dropping funnel and added drop wise to the reaction mixture. The reaction mixture was allowed to come at room temperature and stirred for 6 h. the progress of reaction was monitored by TLC (hexane:ethylacetate, 7:3 v/v). After completion of the reaction, crude solid product was obtained which was filtered and washed with water. The formed product was further re-crystallized by using absolute ethanol. The pure product was obtained which was characterised by FTIR, NMR and melting point.

Yield: 6.26 gm (83%); Mp.: 142-145^{o}C; IR (KBr): 772, 1268, 1438, 1550, 1581, 1651, 1689, 2972, 3228 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.47 (2H, CH_2), 7.32-8.01 (4H, m, ArH), 12.75 (1H, bs, NH); ^{13}CNMR: 43.00, 121, 124, 126, 131, 148, 158, 166; Anal. calc. for $C_9H_7N_2OSCl$: C, 47.69; H, 3.11; N, 12.36; S, 14.15; found C, 47.59; H, 3.08; N, 12.21; S, 14.09.

Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (16a-16i)

A solution of substituted triazole (**12**, 500 mg, 2.6 mmol) in 6 mL dry acetone and triethyl amine (0.4 mL, 2 mmol) were taken in round bottom flask (50 mL). To this N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (**15**, 589 mg, 2.6 mmol) was added. The reaction mixture was stirred at 50^{o}C for 4-5 h. The progress of reaction was monitored by TLC (DCM:methanol, 9:1 v/v). After

completion of reaction, the solvent was removed under reduced pressure and reaction mixture was extracted with ethyl acetate (3×40 mL). The organic layer was dried over anhydrous sodium sulphate and excess of solvent was removed under reduced pressure. The crude product was purified by silica gel (60-120 mesh) column chromatography by using chloroform: methanol (3:1) as eluent to get the desired product which was characterized by FTIR, NMR and melting point.

Yield: 750 mg (75%); Mp.: 242-245^{o}C; IR (KBr): 3342.3229,2957, 1690, 1648, 1546, 1423, 1297, 768 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): δ 2.67 (2H, s, CH_2), 3.50 (1H, s, SH), 7.31-800 (9H, m, ArH), 12.82 (1H, bs, NH); ^{13}CNMR: 36, 121, 122, 123, 124, 126, 129, 132, 163, 165, 167; Anal. calc. for $C_{17}H_{14}N_6OS_2$: C, 53.39; H, 3.69; N, 21.97; S, 16.77; found C, 53.28; H, 3.48; N, 21.89; S, 16.69.

***In-vitro* and docking studies of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives**

Inhibition of acetyl-cholinesterase (AChE) and butyl-cholinesterase (BuChE) activity assay

The N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (16a-16i; Refer Section 3.3) were tested for AChE and BuChE inhibitory activity by using the protocol discussed in Section 2.3 of the Chapter 2.

$$\%\text{AChE/BuChE inhibition} = \frac{(Absorbance\ of\ control - Absrbance\ of\ test) * 100}{Absorbance\ of\ control}$$

Docking studies target molecules against acetyl-cholinesterase (AChE) and butyl-cholinesterase (BuChE)

Geometries of the structures of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (16a-16i; Refer Section 3.3) were optimized at the level B3LYP/STO-3G using Gaussian 09 quantum chemistry software. The structures were verified using vibrational frequencies. Docking of these molecules was performed using the protocol discussed in Section 2.3 of the Chapter 2.

3.4 Results

The inhibition activity of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (16a-16i) against AChE & BuChE are given in figure 3.3. The IC_{50} and docking scores against these two enzymes were provided in Table 3.1.

IC_{50} for AChE **IC_{50} for BuChE**

Figure 3.3: Inhibition activity (IC_{50}) of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives against ACHE and BuChE

Link between numbers in above Figure with molecules represented in Scheme 2

BAT 1 = 16a	BAT 4 = 16d	BAT 7 = 16g
BAT 2 = 16b	BAT 5 = 16e	BAT 8 = 16h
BAT 3 = 16c	BAT 6 = 16f	BAT 9 = 16i

Table 3.1: Docking Score N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (16a-16i)

Compounds	IC_{50}		**Docking Score**	
	AChE	**BuChE**	**AChE**	**BuChE**
BAT 1/16a	967.73	1983.86	-8.165	-9.792
BAT 2/16b	4652.71	1872.32	-6.822	-9.491
BAT 3/16c	578.12	234.15	-7.529	-8.491
BAT 4/16d	326.14	1342.14	-7.060	-9.420
BAT 5/16e	245.19	116.74	-8.915	-9.874
BAT 6/16f	426.56	3784.12	-7.060	-9.420
BAT 7/16g	-	-	Not Docked	Not Docked
BAT 8/16h	2378.34	986.23	-5.951	-7.661
BAT 9/16i	-	-	Not Docked	Not Docked

Chapter 4

Design and synthesis of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives as AChE and BuChE inhibitors

4.1 Introduction

1,2,4 triazole (Fig 4.1) and their derivatives are belong to important class of heterocyclic molecule with known biological activities including antimicrobial,[64] antifungal,[65] anticancer,[66] anti bacterial,[67] anti oxidant,[68] anti inflammatory,[69] anti convulsant activity.[70] The potency and broad spectrum of the pharmacological activity of triazole moiety as neuroprotective agents have attracted the attention of many chemist to explore this scaffold for its potential. Since coumarin and triazole moiety exhibit anti alzheimeric activity (already discussed in Chapter 2 and 3), therefore in this Chapter we synthesize coumarin- triazole conjugate which are evaluated against AChE and BuChE.

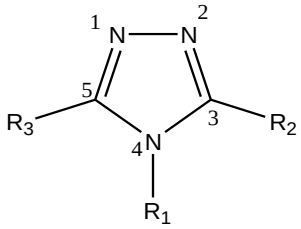


Figure 4.1: Structure of 1,2,4-triazole

4.2 Design of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives and their preliminary *in-silico* analysis of their moieties as AChE and BuChE inhibitor

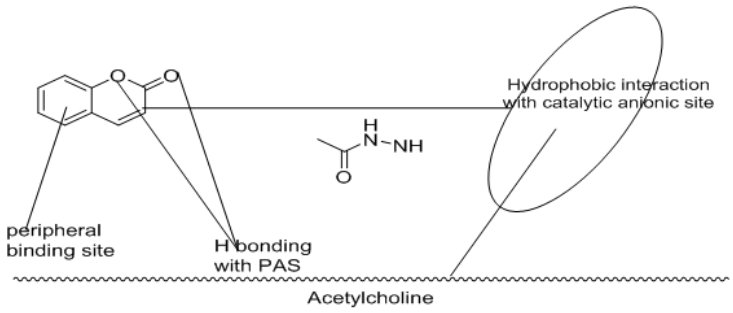
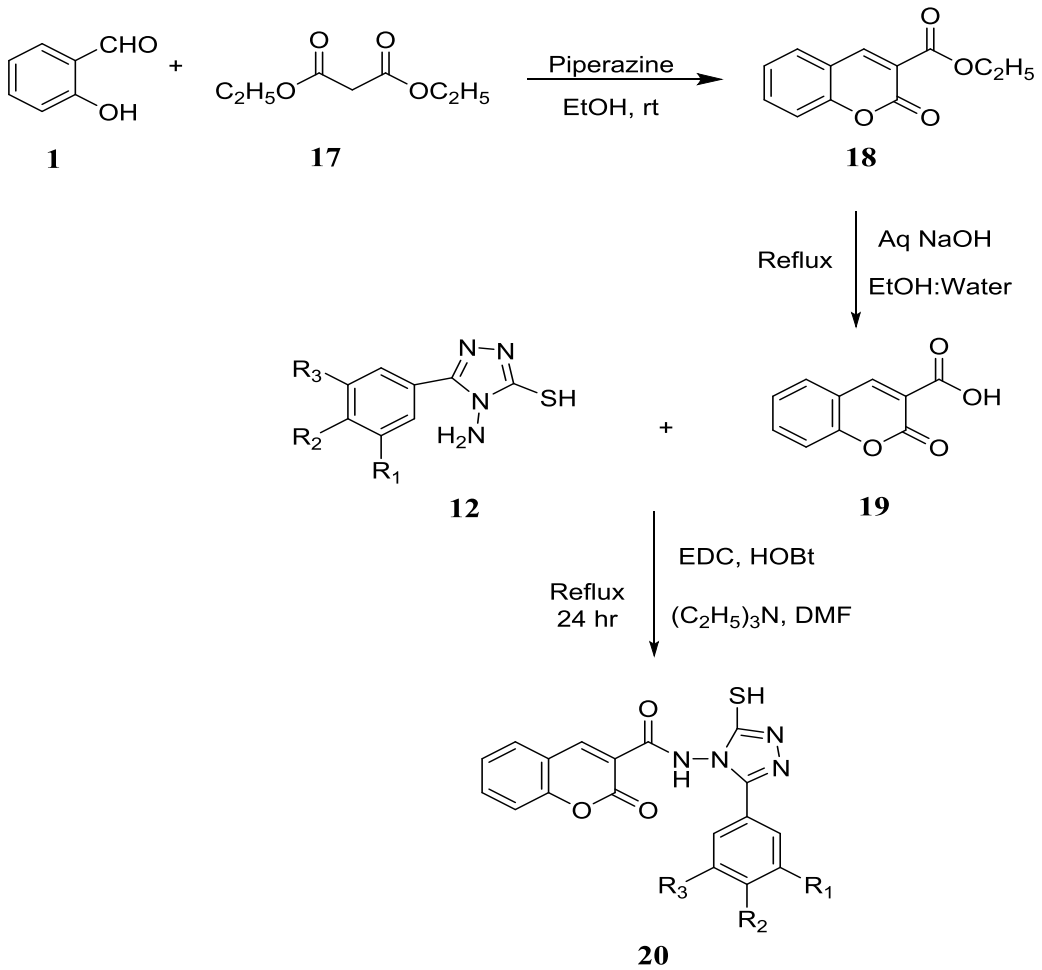


Figure 4.2: Coumarin-triazole conjugate used for design and synthesis potential lead molecules (shown above)

4.3 Experimental Section

The synthesis of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives (**20**) has been achieved by Scheme 4. The synthesized compounds were characterized by FTIR, ^{1}H and ^{13}C NMR, and elemental analysis.



Comp. No.	R_1	R_2	R_3	Comp. No.	R_1	R_2	R_3
20(a)	H	H	H	20(f)	H	CH_3	H
20(b)	H	F	H	20(g)	H	OCH_3	H
20(c)	H	Cl	H	20(h)	H	NO_2	H
20(d)	H	Br	H	20(i)	OCH_3	OCH_3	OCH_3
20(e)	Br	H	H				

Scheme 4: Synthesis of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives

Synthesis of 2-oxo-2H-chromene-3-carboxylic acid ethyl ester (18)

2-oxo-2H-chromene-3-carboxylic acid was synthesized by known procedure.[71]

A solution of diethylmalonate (17, 3.00 mL, 23.5mmol) in 15 mL ethanol was taken in a round bottom flask (250 mL). The solution was kept at 0^{o}C and further peperidine (0.2 mL, 2.0 mmol) was added. The resulting mixture was stirred at 0^{o}C for 5 min followed by addition of salicylaldehyde (1, 2.5 mL, 23.5 mmol). The reaction mixture was allowed to come at room temperature and stirred for 4 hrs. The progress of reaction was monitored by TLC (hexane: ethylacetate, 7:3). After completion of reaction the excess of solvent was removed under reduces pressure and extracted with ethylacetate: dil HCl: water (5:1:4, 3×40 ml). The organic layer was dried over anhydrous sodium sulphate and was concentrated under reduced pressure. The residue obtained was purified by silica gel (60-120 mesh) column chromatography (hexane: ethylacetate, 2:8) as eluent to get the desired product. Colorless solid were obtained which was characterize by melting point.

Yield: 6.98 gm (90%); Mp: 90-94^{o}C

Synthesis of 2-oxo-2H-chromene-3-carboxylic acid (19)

A solution of 2-oxo-2H-chromene-3-carboxylic acid ethyl ester (18, 3 gm, 137.6 mmol) in 6 mL absolute ethanol were taken in round bottom flask (250 mL), and then added aq NaOH (0.5%, 25 mL). The reaction mixture was set for refluxing and refluxed for 5 hrs. The colour of reaction mixture was changes to orange. The progress of reaction was monitored by TLC (chloroform: methanol, 8:2). After completion of reaction HCl was added to make the solution pH =2. Off white precipitate was obtained which was washed with water (3×50 mL). The product was further recrystalized by using absolute ethanol. The molecule was characterized by melting point.

Yield: 2.24 gm (86%); Mp: 188-190^{o}C

Synthesis of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives (20a-20i)

A solution of 2-oxo-2H-chromene-3-carboxylic acid (19, 100 mg, 0.52 mmol) in 3 mL DMF was taken in round bottom flask (25 mL). The solution was kept on ice bath and added HOBt (85.3 mg, 0.52 mmol). The resulting mixture was stirred at 0^{o}C for 15-20 min. After cooling EDC (151 mg, 0.78 mmol) was added. Stirred the reaction mixture further for half an hour white precipitate of EDU was obtained. Then remove the ice bath and substituted triazole (12, 121.2 mg, 0.63 mmol) was added, the reaction mixture was refluxed for 24 hrs. The progress of reaction was monitored by TLC (hexane: ethylacetate, 8:2). After completion of reaction the residue was extracted with ethylacetate (3×50 ml). The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue obtained was purified by silica gel

(60-120 mesh) column chromatography (hexane : ethylacetate, 1:1 as eluent) to get the desired product. The formed product was characterized by IR, NMR and melting point.

Yield 128 mg (67%); Mp.: 188-192^{o}C.

In-vitro and docking studies of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives

Inhibition of acetyl-cholinesterase (AChE) and butyl-cholinesterase (BuChE) activity assay

The 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives (20a-20i; Refer Section 4.3) were tested for AChE and BuChE inhibitory activity by using the protocol discussed in Section 2.3 of the Chapter 2.

$$\%\text{AChE/BuChE inhibition} = \frac{(Absorbance\ of\ control - Absrbance\ of\ test) * 100}{Absorbance\ of\ control}$$

Docking studies target molecules against acetyl-cholinesterase (AChE) and butyl-cholinesterase (BuChE)

Geometries of the structures of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives (20a-20i; Refer Section 4.3) were optimized at the level B3LYP/STO-3G using Gaussian 09 quantum chemistry software. The structures were verified using vibrational frequencies. Docking of these molecules was performed using the protocol discussed in Section 2.3 of the Chapter 2.

4.4 Results

The inhibition activity of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives (20a-20i) against AChE & BuChE are given in Figure 4.3. The IC_{50} and docking scores against these two enzymes were provided in Table 4.1.

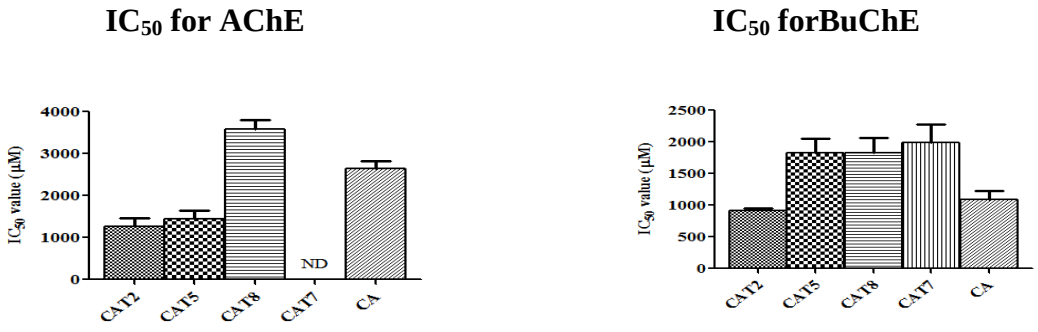


Figure 4.3: Inhibition activity (IC_{50}) of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives against ACHE and BuChE

Link between number in above Figure with molecules represented in Scheme 4

CAT 1 = 20a	CAT 4 = 20d	CAT 7 = 20g
CAT 2 = 20b	CAT 5 = 20e	CAT 8 = 20h
CAT 3 = 20c	CAT 6 = 20f	CAT 9 = 20i

Table 4.1. Docking Score of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives (20a-20i)

Compounds	**IC_{50}**		**Docking Score**	
	AchE	**BuChE**	**AchE**	**BuChE**
CAT1/20a	1156.23	987.73	-7.564	-7.960
CAT2/20b	1201.23	1859.24	-6.377	-10.250
CAT3/20c	-	-	-5.775	-10.789
CAT4/20d	-	-	-5.970	-10.357
CAT5/20e	3738.69	1866.12	-6.475	-9.892
CAT6/20f	-	-	-5.496	-10.383
CAT7/20g	375.14	2345.20	-6.466	-9.457
CAT8/20h	-	-	-5.445	-9.389
CAT9/20i	-	-	-5.955	-8.123

Chapter 5

Conclusions

- On the basis of hypothesis, we have synthesized total 106 molecules including 40 novel molecules.
- Docking analysis and *in-vitro* analysis can provide data that facilitates SAR (structure activity relationship) analysis for inhibitors of AChE and BuChE.
- *In silico* docking results are consistent with *in vitro* IC_{50} for BuChE. Whereas high similar docking scores were obtained for all diverse molecules against AChE.
- Based on the IC_{50} value (*In vitro* studies), the synthesized compounds showed less to no activity towards AChE, but a remarkably high activity against BuChE. Molecule with IC_{50} value of 100 μM or less is considered as a good lead molecule.
- Out of the synthesized molecules, compound from Chapter 2; 8b, 8j, 8i; from Chapter 3; 16c, 16e, 16f; and from Chapter 4; 20f, 20g and 20h, exhibit activity towards BuChE/AChE. Many of them are selective against either of the enzyme.

Future prospects

- From these generated data, SAR can be used for designing of new lead molecules which are expected to provide activity in μM/nm range.
- New target specific molecule can be designed which is based on thiazole and triazole moieties.
- Molecular simulation methods may be used to design better potent drugs.

Publications:

Patent filed– 01

1. Ram Singh, **Deepak Mishra**, Atiya Fatima, Chittaranjan Rout, Vineet Mehta, Udayabanu Malairaman, Mamta Chaudhary; Novel Thiazole Compounds, Process For Preparing The Same and Pharmaceutical Formulation Thereof. Ref No PAT134/003/17 dated 11-07-2017.

Referred Journal – 03

1. **Deepak Mishra**, Atiya Fatima, Chittaranjan Rout and Ram Singh; An efficient one-pot synthesis of 2-Aminothiazole Derivatives; *Der Chemica Sinica*, 6(8), 14-18, **2015**. [*ISSN* 0976 – 8505, IF = 0.676]
2. **Deepak Mishra**, Ram Singh and Chittaranjan Rout; A facile amidation of chloroacetyl chloride using DBU; *International Journal of ChemTech Research*, 10(3), 365-372, **2017**. [*ISSN*: 0974-4290 IF = 0.598 (2014)]. (Scopus indexed)
3. **Deepak Mishra**, Ram Singh and Chittaranjan Rout; Synthesis of highly functionalized pyrazoles using $AlCl_3$ as catalyst; *Journal of Chemical and Pharmaceutical Research*; 9(6), 16-19, **2017**. [ISSN 0975-7384, IF = 0.467, 2014] (Scopus indexed)

Conferences – 05

1. **D. Mishra**, Geetanjali, C. Rout, and R. Singh; Synthesis of novel Thiazole Derivatives as potential Anti-Alzheimeric Agents; National Symposium on "Chemistry at the interface of Innovative Researchers in Science and Technology" held on 27-28 February **2014**, organized by Department of Chemistry, University of Allahabad.
2. A. Fatima, **D. Mishra**, C. Rout and R. Singh; Synthesis and Characterization of Isoflavone based molecule as Antiestrogens; Indian Roadshow Workshop - 4 November **2014** organized by Royal Society of Chemistry at IIT Delhi, India.
3. **D. Mishra**, Geetanjali, C. Rout and R. Singh; Synthesis and Characterization of selected Imidazole Derivatives as potential Anti-Alzheimeric Agents; The 102nd *Indian Science Congress* 2015 in association with the University of Mumbai, 3-7 January **2015**.
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5. **D. Mishra**, Babita, C. Rout and R. Singh; Synthesis of selected Triazole Derivatives as potential Anti-Alzheimeric Agent; 6th International symposium entitled CTDDR 2016" held on 25-28 February **2016**, organized by CDRI , Lucknow, India.

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